

Epidemiological Survey Of Antimicrobial-resistant *Salmonella* In Market-age Swine

Harvey RB¹, Farrington LA², Buckley SA¹, Droleskey RE¹, Stanker LH¹, Nisbet DJ¹, Inskip PD²

¹ Food Animal Protection Research Laboratory, USDA, ARS, College Station, TX 77845 USA

² Dept. of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843 USA

Summary

We conducted a survey of antibiotic resistance in *Salmonella* isolated from market-age swine at five different farms. These farms, which were sampled between October 1997 and June 1998, were part of a completely integrated Texas swine operation. Four of the farms sampled were farrow-to-finish farms and were sampled three times each. The fifth farm provided replacement gilts for the other farms in the operation and was sampled once. Samples were taken from the lymph nodes and cecal contents at the time of slaughter. Of the 559 *Salmonella* isolates recovered, 420 were sent to the National Veterinary Services Laboratory for serotyping. Resistance patterns were determined by disk diffusion using thirteen antibiotics, and 320 isolates were analyzed. Resistance was observed to ampicillin, chloramphenicol, chlortetracycline, nitrofurantoin, penicillin G, streptomycin and sulfisoxazole. Only 21 isolates (6.9%) demonstrated no resistance to any of the antibiotics tested. Multidrug resistance (resistance to two or more antibiotics) was observed in 183 isolates (57.2%) with 34 of these isolates (18.6%) resistant to three antibiotics and 26 (8.1%) resistant to four antibiotics. The most common three drug resistance pattern consisted of chlortetracycline, penicillin G and streptomycin (37.3%). All four drug resistance patterns consisted of chlortetracycline, penicillin G, streptomycin and sulfisoxazole (100%). A significant difference was observed between serotypes and between somatic serogroups in their antibiotic resistance patterns. Variation also was observed between farms. As a step in understanding the connection between antibiotic use in agriculture and medicine and emergence of antibiotic resistant bacteria, programs that monitor the levels of antibiotic resistance must be continued. In animal production, where subtherapeutic administration of antibiotics can be common, continued surveillance is especially important.

Introduction

The issue of antibiotic resistance in bacteria has raised concerns for health and safety worldwide. Resistance was first noted in 1940 when an enzyme capable of hydrolyzing penicillin was detected in *E. coli* (10). Since that time antibiotic resistance has become common in many genera of bacteria (9,10,12). Today many *Salmonella* serotypes have been isolated that demonstrate resistance to multiple antibiotics (3,4,11). *S. typhimurium* strain DT104, which is found in many places including the United States and Europe, has demonstrated resistance to ampicillin, tetracycline, streptomycin, chloramphenicol and sulfonamides (5,12). Resistance to antibiotics can create problems in both human and veterinary medicine (1-3). For example, if a *Salmonella* infection becomes systemic, serious illness and even death can occur if the antibiotics selected for treatment are not effective due to resistance by the bacteria (3). Vast amounts of antibiotics are used each year, not only in the treatment of illness in humans and animals, but also subtherapeutically in food-producing animals for prophylaxis and growth promotion (3,12). In the early 1980's approximately 14.3 million kg of antibiotics were produced annually in the United States, of which about 40% was used as a feed additive for livestock and poultry (9). It has been theorized that the widespread use of antibiotics in human and veterinary medicine, as well as in animal production, has acted as a selective agent for antibiotic resistance in bacteria (3,4,9,10,12). This study was initiated to determine the extent of antibiotic resistance in various *Salmonella* serotypes isolated from swine and to identify the resistance patterns that occur.

Materials and Methods

All media, antibiotic disks and test reagents used in this study were manufactured by Difco with the exception of the brilliant green agar (Oxoid).

Sample Collection

Ileo-cecal lymph nodes and cecal contents were collected from market-age swine at the time of slaughter from October 1997 to July 1998. Sampling occurred multiple times at each of five farms (farms A-E), which were part of a completely integrated, Texas swine operation. Four of the farms sampled were farrow-to-finish farms and one farm was a gilt replacement farm. Pre-enrichment for lymph nodes and cecal contents was performed in both GN Hajna broth and tetrathionate broth base with added iodine. This generated four samples (two lymph node and two cecal content samples) per pig. Following post-enrichment of all samples in Rappaport-Vassiliadis R10 broth, each sample was streaked on brilliant green agar with novobiocin for identification. Samples that displayed typical colony morphology for *Salmonella* were characterized biochemically using lysine iron agar and triple sugar iron agar. *Salmonella* positives were confirmed by slide agglutination using *Salmonella* O antiserum poly A-I and Vi and Group C1 factors 6,7. Each sample was stored in pure culture on tryptic soy agar.

Antimicrobial Resistance – Disk Diffusion

The prevalence of antimicrobial-resistant *Salmonella* was determined by disk diffusion for thirteen antibiotics commonly used in human and veterinary medicine following guidelines published by the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 1997; NCCLS, 1998). The antibiotics tested were amikacin (30µg), ampicillin (10µg), cephalothin (30µg), chloramphenicol (30µg), chlortetracycline (30µg), enrofloxacin (5µg), gentamicin (10µg), kanamycin (30µg), nitrofurantoin (300µg), penicillin G (10 units), streptomycin (10µg), sulfisoxazole (300µg) and trimethoprim (5µg). These antibiotic concentrations are recommended by the NCCLS and are based on normal dose-range schedules (NCCLS, 1997). Each *Salmonella* isolate was grown overnight on blood agar. Five ml of tryptic soy broth was inoculated with four to five *Salmonella* colonies to achieve turbidity similar to that of a 0.5 McFarland standard (direct colony suspension method). This suspension was streaked on a 150mm Mueller Hinton plate, and the antibiotic disks were placed on the plate using a 12-cartridge semi-automatic dispenser. The thirteenth antibiotic disk was placed manually in the center of the plate. The plates were incubated at 37°C for 18-20 hours. For each antibiotic disk, the zone of inhibition was measured as the diameter of the circle without bacterial growth around that disk. Each isolate was classified as susceptible, intermediate or resistant to each of the thirteen antimicrobial agents using the NCCLS zone diameter interpretive standards for

Enterobacteriaceae based on clinical efficacy (NCCLS, 1998).

Data Analysis

For the purpose of statistical analysis, intermediate resistance was considered a degree of resistance, and these isolates were placed in the resistant category. All statistical analysis was performed using Fisher's Exact Test.

Results and Discussion

Table 1. Multidrug Resistance of *Salmonella* Isolates

# of Antibiotics Resistant to	# Resistant	% Resistant
4	26	8.1%
3	34	10.6%
2	123	38.4%
1	116	36.3%
0	21	6.6%

Of the 559 *Salmonella* isolates recovered, 420 were sent to the National Veterinary Services Laboratory for serotyping and 320 isolates were analyzed by disk diffusion using thirteen antibiotics (Figure 1). Resistance was found to several antimicrobial agents used extensively in the past. Eighty-six percent of the isolates were resistant to penicillin G, an antibiotic introduced in both human and veterinary medicine in the 1940's and still used today primarily in large animal medicine (1,6). Ampicillin and cephalothin, both related to penicillin, are often active against penicillin G-resistant bacteria (6). Ampicillin resistance was at 1% and intermediate resistance to ampicillin was 1%, whereas there was 0% resistance to cephalothin. Reduced sensitivity (61% resistant, 39% intermediate) to chlortetracycline was seen in 100% of the samples. Chlortetracycline was first introduced into veterinary medicine in 1948 and now is used as a feed and water additive for food-producing animals (1). Resistance also has developed against the aminoglycoside streptomycin, which was first used in human and veterinary medicine in the 1940's (6). Seventeen percent of the samples were classified as resistant to streptomycin and 62% of the samples were classified as intermediate. Virtually no resistance was observed to the three other aminoglycosides, kanamycin, gentamicin, and amikacin. Only 1% of the samples were intermediate to kanamycin. Sulfisoxazole, a sulfonamide, has been used clinically for about 50 years. It is now used almost exclusively in combination with another drug, such as trimethoprim, in both human and veterinary medicine (1,6).

Resistance to sulfisoxazole was observed with 12% of the samples considered resistant and 12% of the samples considered intermediate. No resistance to trimethoprim was seen. Chloramphenicol, introduced in 1947, and nitrofurantoin, introduced in 1953, have been banned from use in food-producing animals in the United States, but are still used in human and companion animal medicine (1,2,6). One percent of the samples were resistant to chloramphenicol. Two percent of the samples were resistant and 1% of the samples were intermediate to nitrofurantoin. No resistance was observed to the fluoroquinolone, enrofloxacin.

Multidrug-resistance (resistance to two or more antibiotics) was found in 57.1% of the samples. Resistance to two, three, and four antibiotics was found in 38.4%, 10.6%, and 8.1% of the samples, respectively (Table 1). Seventeen of the 26 isolates (65%) resistant to four antibiotics were *S. agona* and 9 of the 26 (35%) were *S. derby*. Both *S. agona* and *S. derby* are somatic serogroup B. All 26 were resistant to the same four antibiotics—chlortetracycline, penicillin G, streptomycin and sulfisoxazole. Out of the 10.6% (34 samples) resistant to three antibiotics, all were resistant to chlortetracycline, penicillin G and either streptomycin, sulfisoxazole, or ampicillin. The antibiotic resistance patterns by serotype and somatic serogroups can be seen in Tables 2 and 3. A significant difference ($P<.05$) was found between serotypes in their antibiotic resistance patterns, with *S. agona*, *S. derby*, *S. livingstone*, *S. montevideo*, and *S. schwarzengrund* showing increased resistance to streptomycin, and *S. agona*, *S. derby*, *S.*

schwarzengrund and the "other" category showing increased resistance to sulfisoxazole when compared to the remaining serotypes. A significant difference ($P<.05$) also was detected between somatic serogroups in their resistance patterns. Somatic serogroups B and C₁ expressed increased resistance to streptomycin, and somatic serogroup B expressed increased resistance to sulfisoxazole. The "other" category showed increased resistance to ampicillin, but showed decreased resistance and increased intermediate susceptibility to chlortetracycline when compared to the remaining serogroups.

A significant difference ($P<.05$) was detected among farms in resistance of the *Salmonella* spp. isolated to the antibiotics chlortetracycline and sulfisoxazole (data not shown). The highest rate of resistance to chlortetracycline was found in the samples from farm A, and the lowest rate of resistance was found in the samples from farm E (gilt replacement). All of the farms sampled in this study infrequently administered chlortetracycline as a therapeutic agent. Subtherapeutic administration of chlortetracycline was discontinued on all of the farms approximately five years prior to this study.

As a step in understanding the connection between antibiotic use in agriculture and medicine and emergence of antibiotic resistant bacteria, programs that monitor the levels of antibiotic resistance must be continued. In animal production, where subtherapeutic administration of antibiotics is common, continued surveillance is especially important.

Table 2. Antimicrobial Resistance of *Salmonella* by Somatic Serogroup^a

Antibiotic ^b	B (145)	C ₁ (97)	E ₁ (37)	Other ^c (41)	p-value ^d
Ampicillin	1%	0%	0%	7%	.014
Chlortetracycline	72%	62%	43%	39%	<.001
Streptomycin	87%	82%	57%	63%	<.001
Sulfisoxazole	41%	6%	14%	15%	<.001

^aPercent of resistant isolates for each serogroup

^bThe only antibiotics shown are those in which the p-value approaches significance

^cThe "other" category represents somatic serogroups that occurred less than 15 times in the 320 samples analyzed. These serogroups and the number of times they each occurred are as follows: C₂(7), D₁(12), E(2), E₂(1), E₃(1), G₂(6), N(1), R(1), and an undefined group containing the 4,5,12:I-monophasic and multiple serotypes classifications that have no defined somatic serogroup.(10)

^dFisher's exact test for association between serogroups and antibiotic resistance.

Table 3. Antimicrobial Resistance of *Salmonella* by Serotype^a

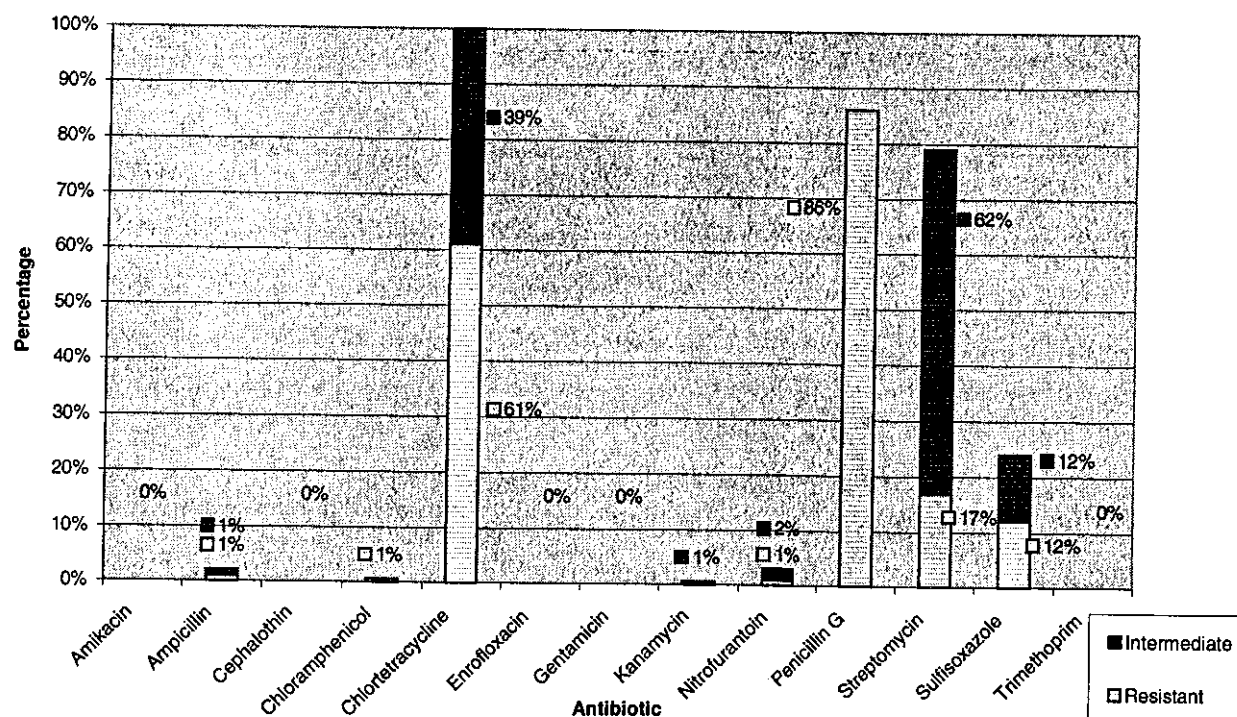
Antibiotic ^b	Agona (35)	Derby (21)	Livingstone (37)	Montevideo (46)	Schwarzengrund (60)	Other ^c (121)	p-value ^d
Chlortetracycline	91%	86%	73%	52%	80%	39%	<.001
Penicillin G	69%	81%	78%	89%	97%	87%	.003
Streptomycin	94%	86%	84%	87%	90%	64%	<.001
Sulfisoxazole	60%	67%	0%	2%	37%	16%	<.001

^aPercent of resistant isolates for each serotype.

^{b,d}See footnotes on Table 2 .

^cThe "other" category represents serotypes that occurred less than 20 times in the 320 samples analyzed. These serotypes and the number of times they each occurred are as follows: 4,5,12:I-monophasic(6), *S. braenderup*(4), *S. havana*(5), *S. johannesburg*(1), *S. mbandaka*(3), *S. meleagridis*(3), *S. menhaden*(1), *S. muenchen*(2), *S. muenster*(13), multiple serotypes(2), *S. newbrunswick*(1), *S. orion*(2), *S. tennessee*(1), *S. thompson*(4), *S. typhimurium*(15), *S. typhimurium* var. *copenhagen*(10), *S. urbana*(1), *S. worthington*(1), 3,10:L,W-monophasic(2), *S. anatum*(18), *S. heidelberg*(4), *S. infantis*(2), *S. javiana*(12), *S. newport*(5), *S. uganda*(1), and untypable(2)

Figure 1: Percentage of Isolates Classified as Resistant and Intermediate to Each Antibiotic



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